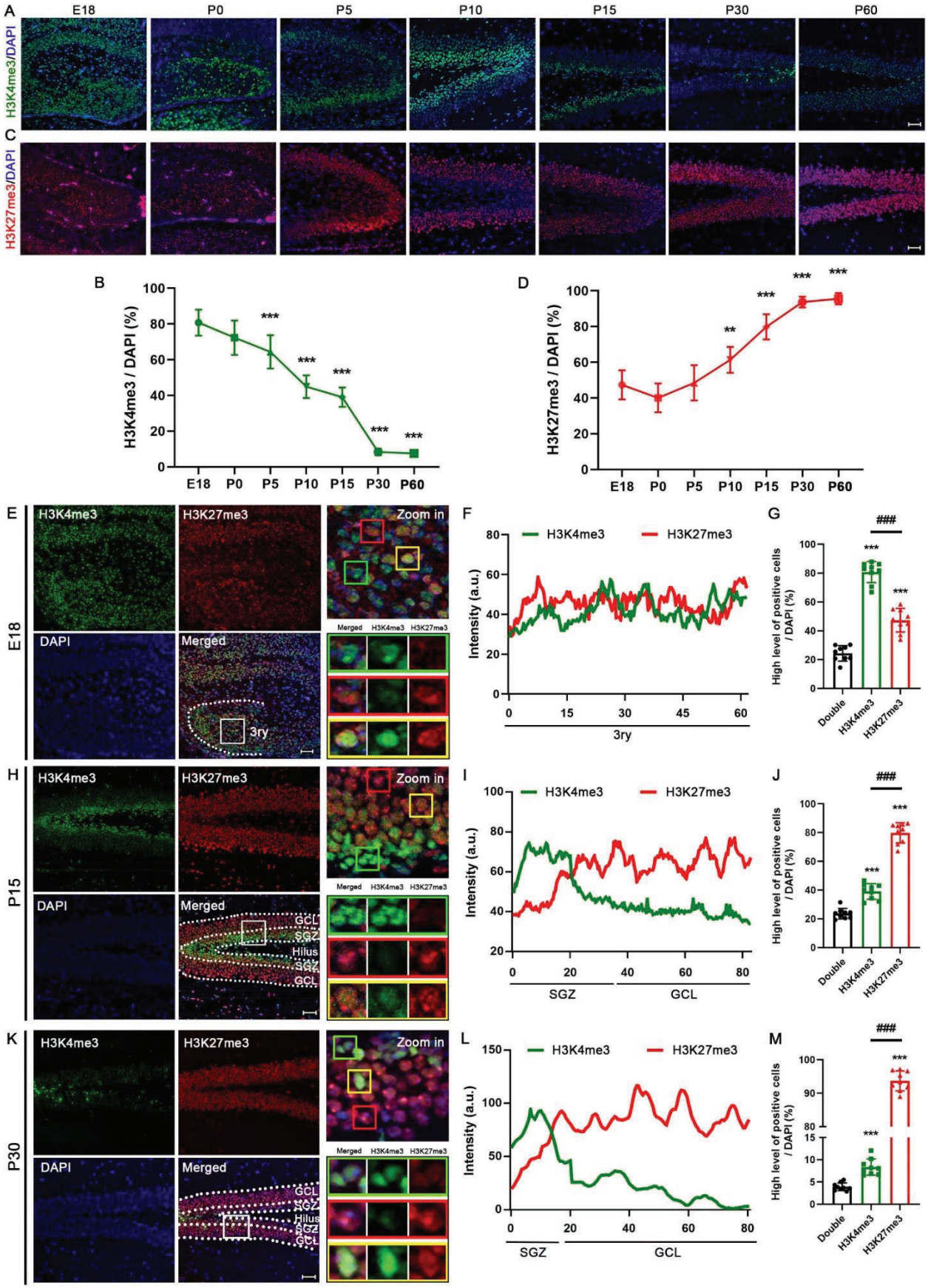
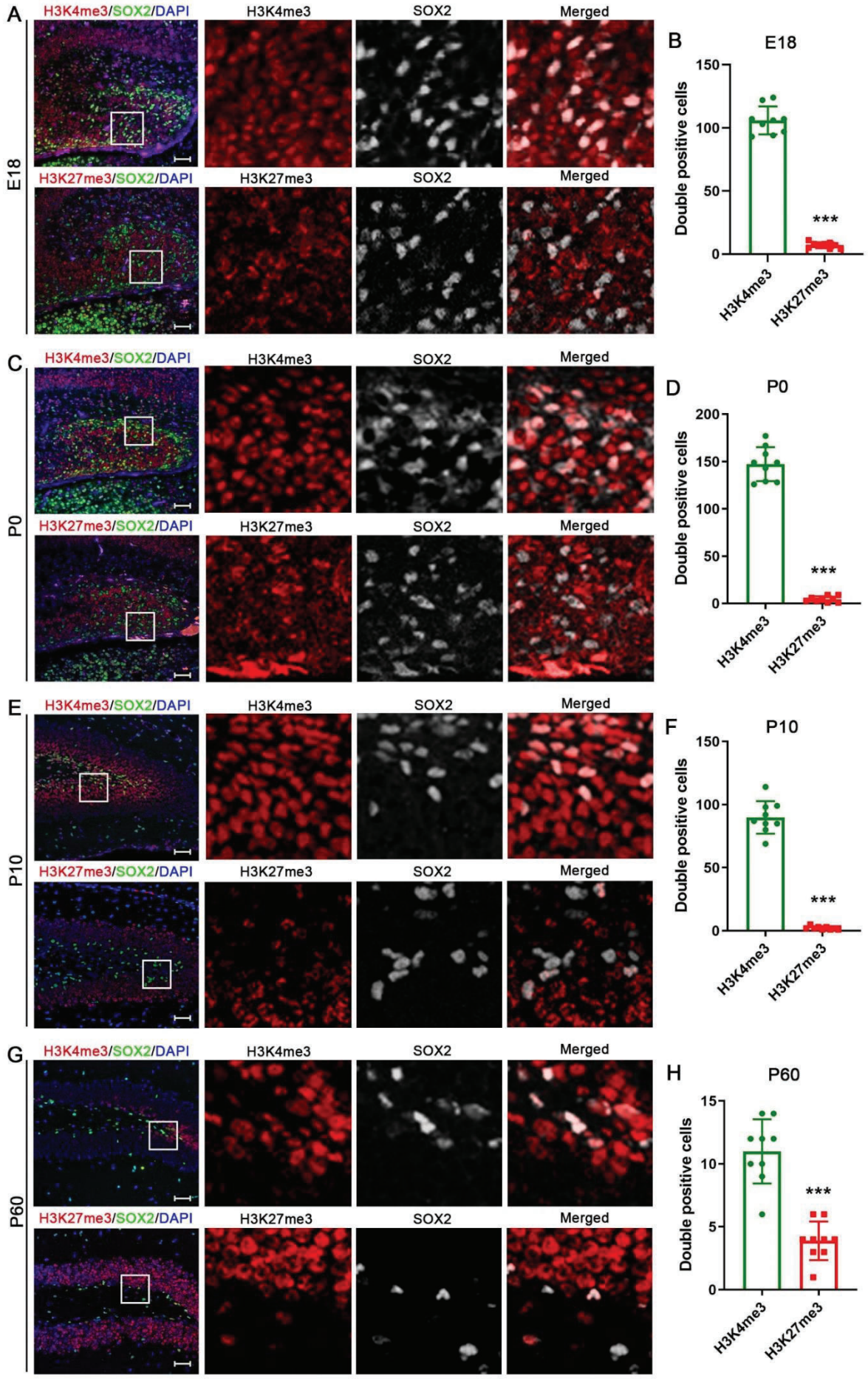
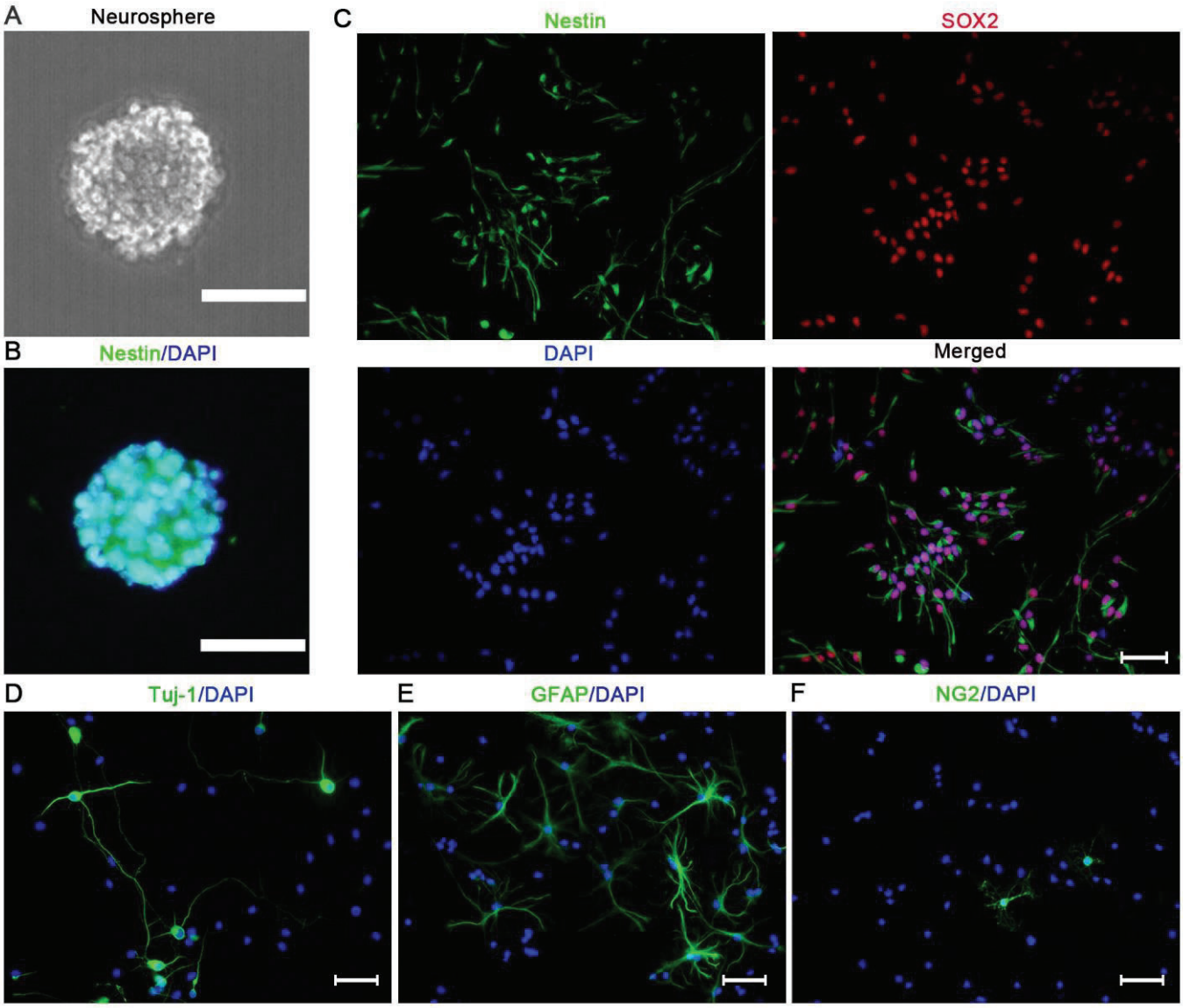


Supplement Figure 1. The levels and distribution of H3K4me3 and H3K27me3 exhibit significant dynamic changes during dentate gyrus development.

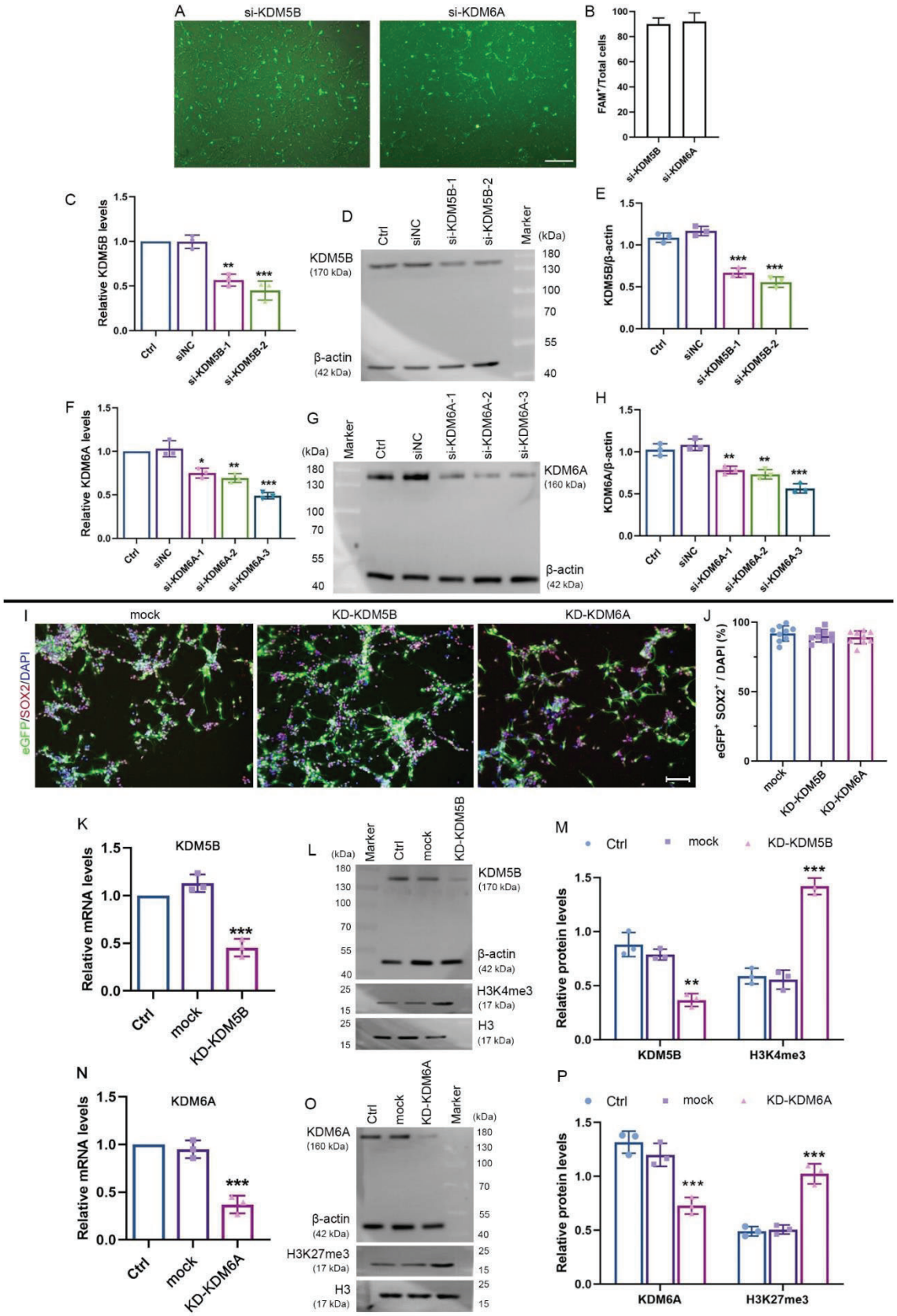
H3K4me3 (A) and H3K27me3 (C) were detected by immunofluorescence staining from E18 to P60 in dental gyrus. (B, D) The number of immunolabeled cells was counted and data are presented as the mean ± standard deviation of three independent experiments (n = 3). $^{**}P < 0.01$, $^{***}P < 0.001$ versus E18 group. (E, H and K) H3K4me3 and H3K27me3 colocalization shown by two-colour immunofluorescence staining at different periods of dental gyrus development. Nuclei were counterstained with DAPI. The square frames are enlarged to identify a single typical of H3K4me3 (green), H3K27me3 (red) and double positive cells (yellow). (F, I and L) The fluorescence intensity of H3K27me3 and H3K4me3 is presented. (G, J and M) The number of immunolabeled cells was counted and data are presented as the mean ± standard deviation of three independent experiments (n = 3). $^{***}P < 0.001$ versus double group; $^{\#\#\#}P < 0.001$ versus H3K4me3 group. E18, embryo at day 18; P0, neonatal mouse; P5, postnatal at day 5; P10, postnatal at day 10; P15, postnatal at day 15; P30, postnatal at day 30; P60, postnatal at day 60; 3ry, tertiary germinal matrix; GCL, granule cell layer; SGZ, subgranular zone. Scale bar = 50 μm.



Supplement Figure 2. High level of H3K4me3 co-located with SOX2 during neurodevelopment in dental gyrus.
Immunocytochemical double labeling revealed H3K4me3 and H3K27me3 co-stained with SOX2 at E18 (A), P0 (C), P10 (E), and P60 (G) in dentate gyrus. The magnified view of the boxed region is shown on the right. Nuclei were counterstained with DAPI. (B, D, F and H) The number of immunolabeled cells was counted and data are presented as the mean ± standard deviation of three independent experiments (n = 3). ***$P <$ 0.001 versus H3K4me3 group. E18, embryo at day 18; P0, neonatal mouse; P10, postnatal at day 10; P60, postnatal at day 60. Scale bar = 50 μm.

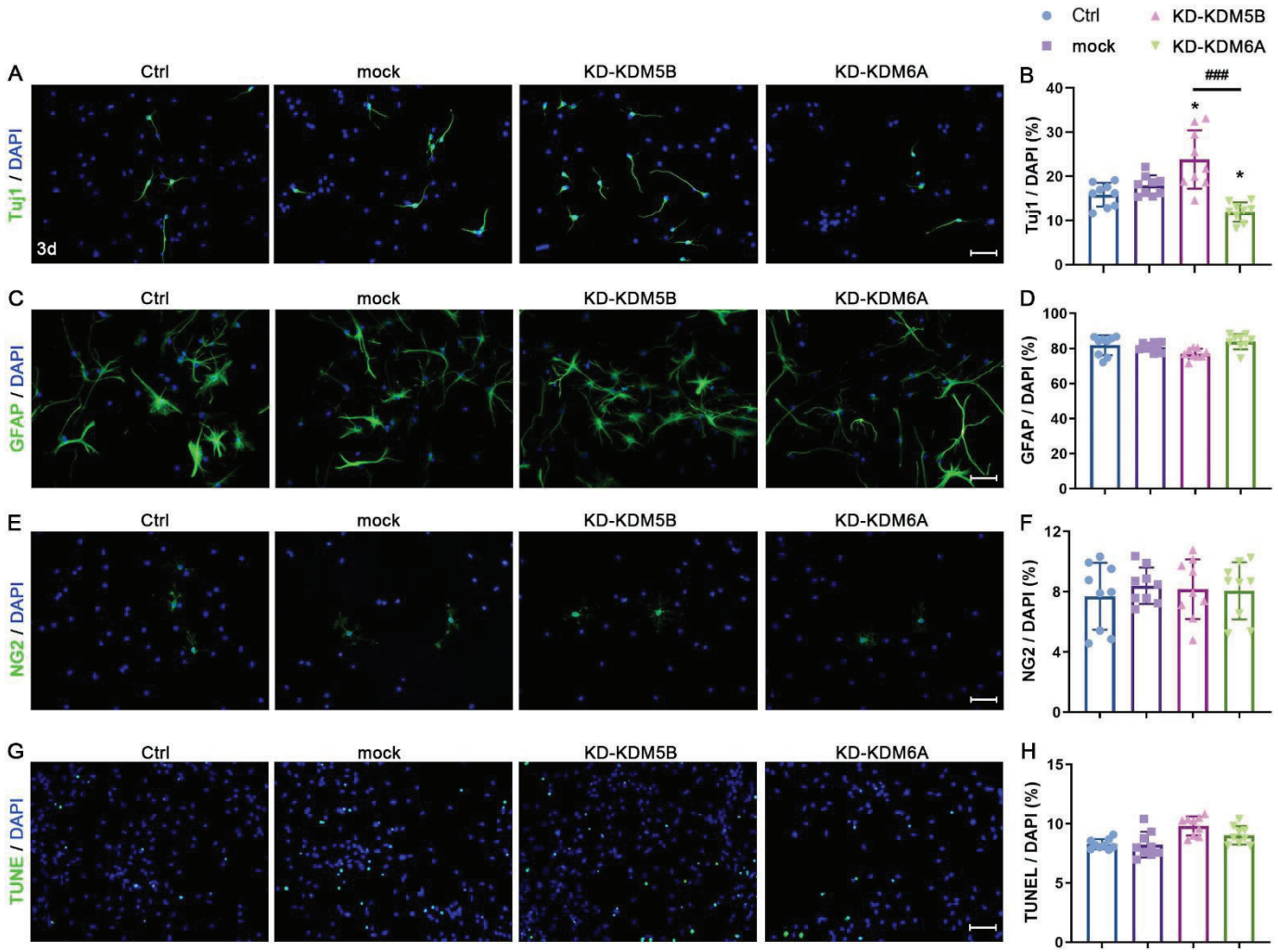


Supplement Figure 3. Culture and immunostaining characterization of neonatal mouse hippocampal NSCs.
Following a 3 to 5 days incubation period, neurospheres with a diameter ranging from 80–230 μm were observed (A), with the majority of these cells expressing nestin (B). (C) Double-positive cells for SOX2 and Nestin were detected in adherent cultured hippocampal NSCs. Upon culturing in the natural differentiation medium for 5 days, Tuj-1 (D), GFAP (E) and NG2 (F) positive cells were observed. Scale bars in A, B are 100 μm; in C-F are 50 μm.

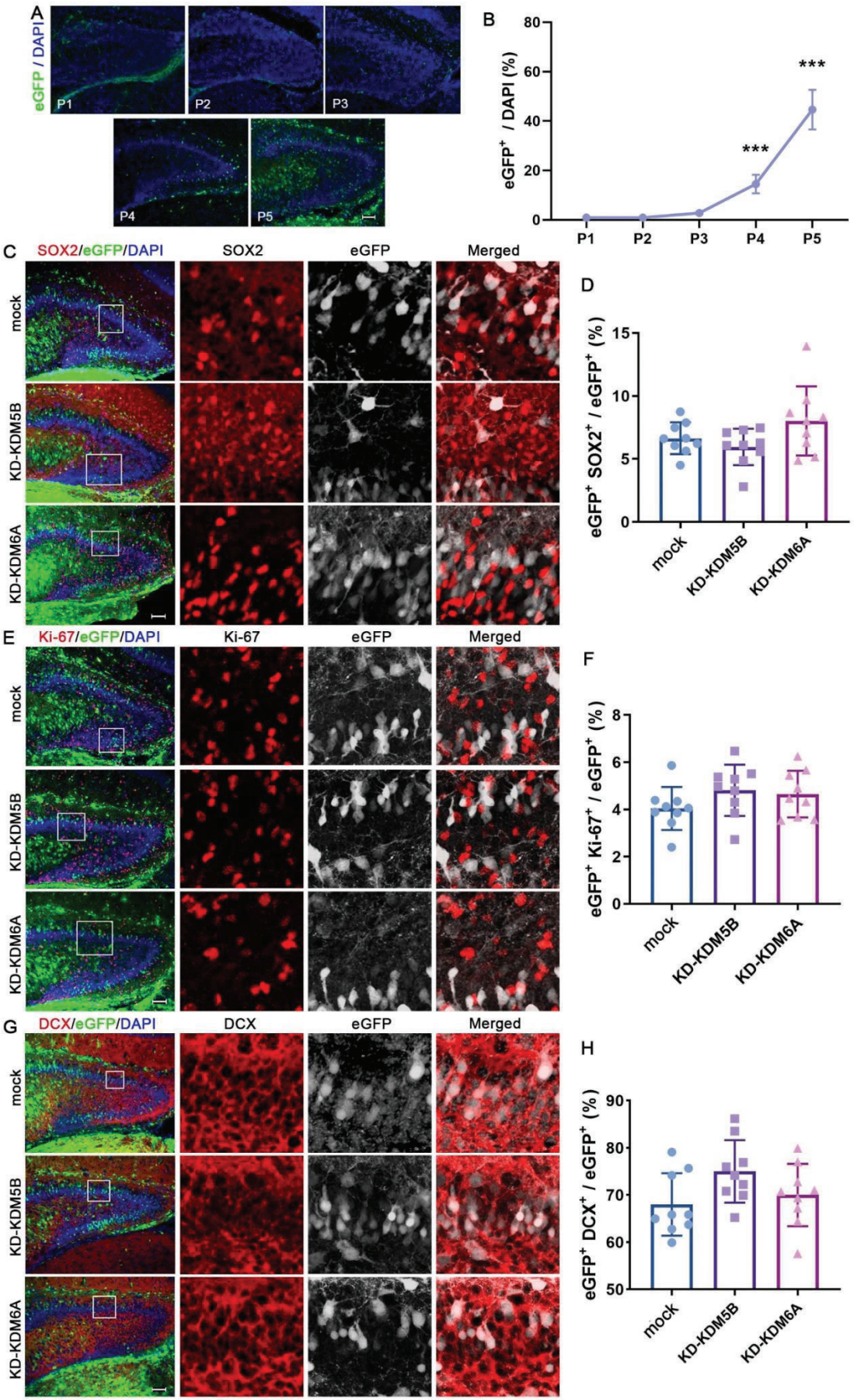


Supplement Figure 4. KDM5B and KDM6A-targeted shRNA effectively reduces their expression in cultured neonatal mouse hippocampal NSCs.

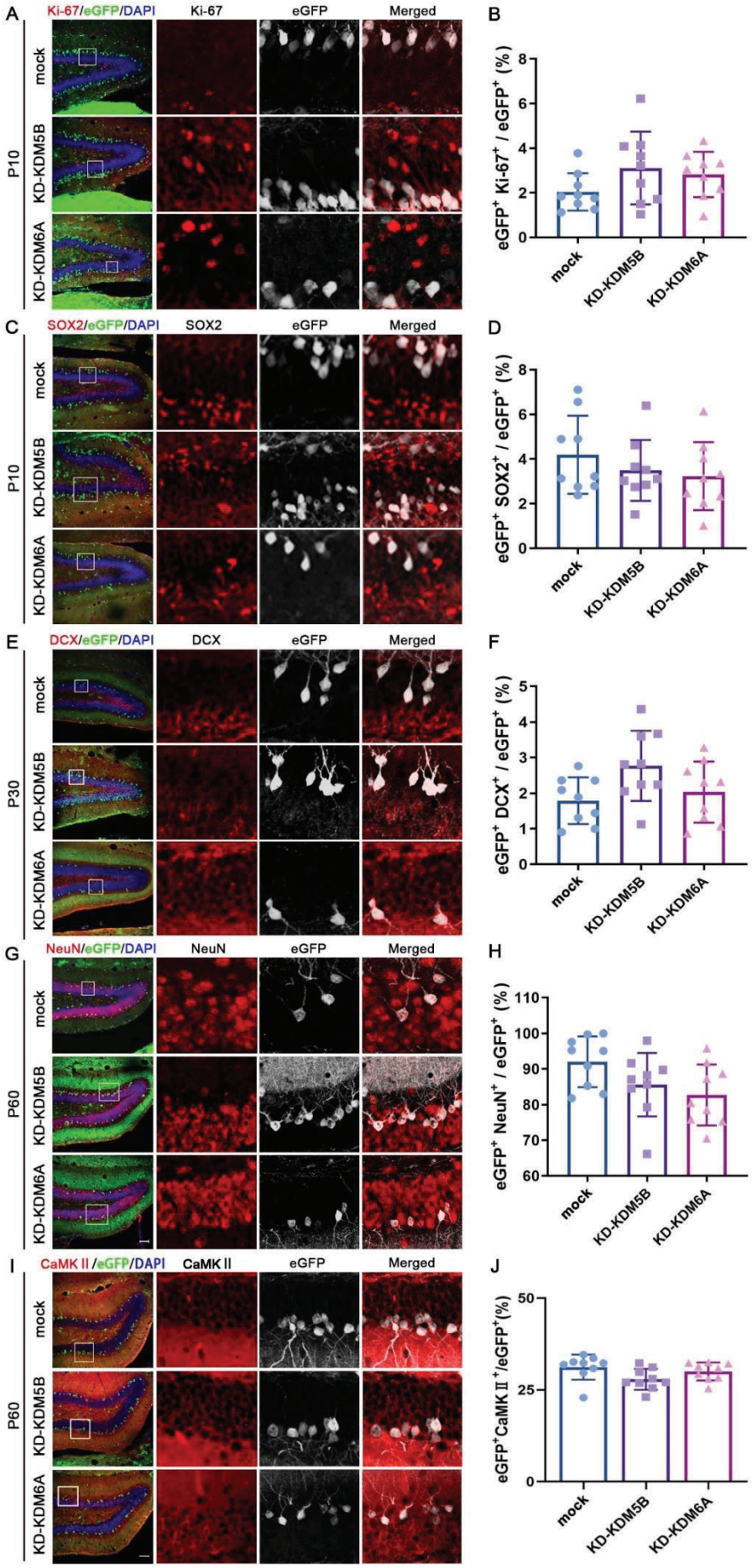
(A, B) NSCs were transfected for 6 h with FAM-labeled non-specific siRNAs (siNC), KDM5B-targeted siRNA (si-KDM5B-1 & 2) or KDM6A-targeted siRNA (si-KDM6A-1, 2 & 3) by using Lipofectamine 2000. An equal volume of medium was added to the control group. Six hours later, more than 90% of transfected cells were observed (green). Scale bar = 200 μm. The expression of KDM5B and KDM6A was detected by qRT-PCR (C, F) and Western blot (D, E, G and H), respectively. The presented data were from three independent experiments (n = 3) and were expressed. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus the siNC group. The sequences si-KDM5B-2 (KD-KDM5B) and si-KDM6A-3 (KD-KDM6A) were used for the generation of lentivirus. Empty vector was used as a lentiviral empty vector control (mock). NSCs were infected with eGFP-labeled lentivirus and selected with puromycin (1.6 μg/mL). (I, J) After 3 days of culturing, more than 90% of GFP-labeled cells were co-stained with SOX2. The presented data were from three independent experiments (n = 3). Scale bar = 100 μm. (K, N) The expression levels of KDM5B and KDM6A were assessed using qRT-PCR. The presented data were obtained from three independent experiments (n = 3). $^{***}P < 0.001$ versus the mock group. (L, O) Additionally, the expression of KDM5B, H3K4me3, KDM6A, and H3K27me3 was evaluated through Western blot analysis. (M, P) The presented data were obtained from three independent experiments (n = 3). $^{**}P < 0.01$, $^{***}P < 0.001$ versus the mock group.



Supplement Figure 5. H3K4me3 and H3K27me3 have minimal impact on the glial differentiation.
The neonatal mice hippocampal NSCs were stably transfected with mock, KD-KDM5B and KD-KDM6A, respectively. (A) Tuj1-positive cells were identified by immunostaining after 3 days of culturing in a natural differentiation medium. Likewise, GFAP (C) and NG2 (E) positive cells underwent immunostaining in the same medium. (G) The apoptotic cells were detected by TUNEL assay. Scale bar = 50 μm. (B, D, F and H) Data are presented as the mean ± standard deviation of nine independent experiments (n = 9). $^{*}P < 0.05$ versus mock group. $^{\#\#\#}P < 0.001$ versus KD-KDM5B group.

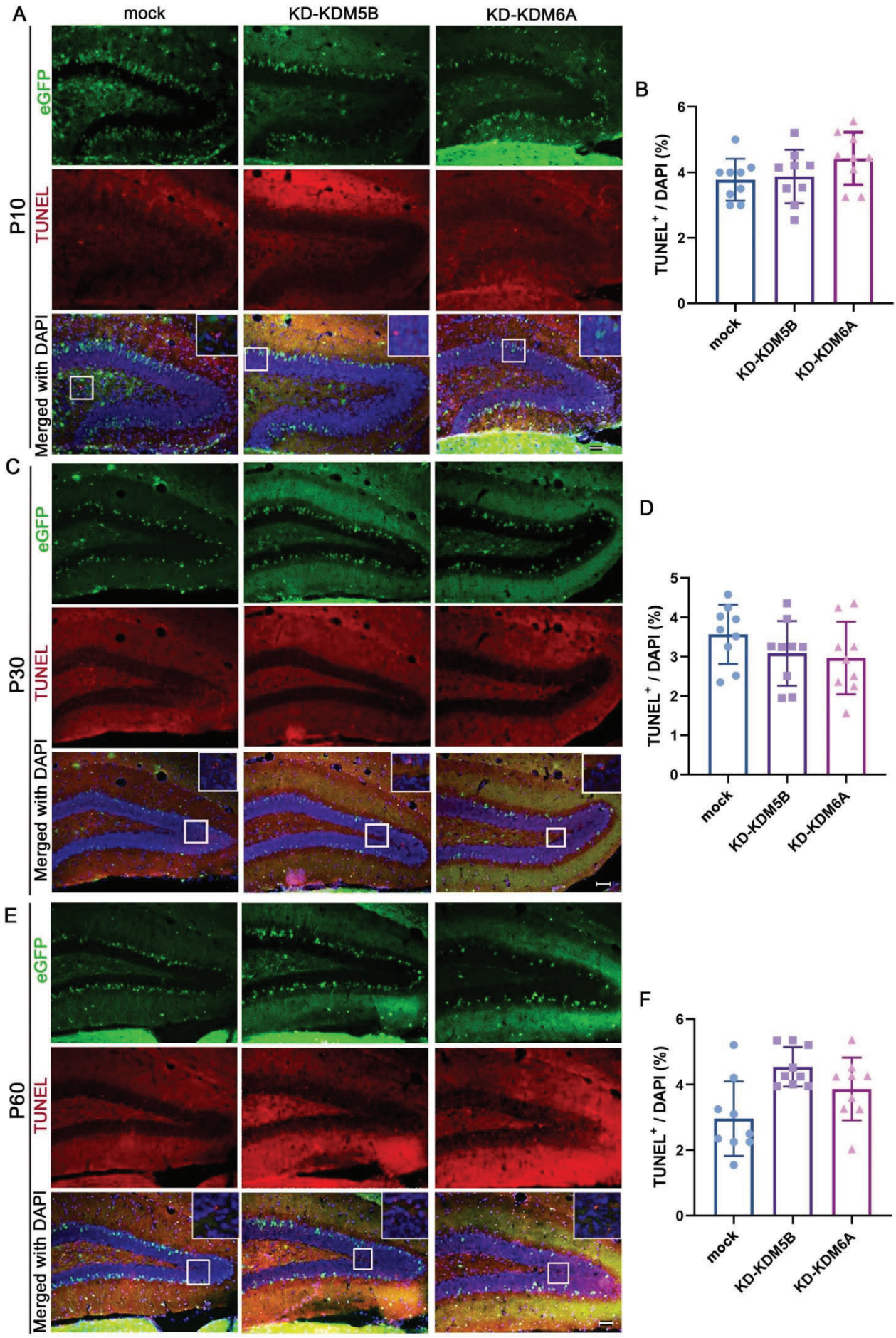


Supplement Figure 6. Recombinant adeno-associated virus 9-labeled cells rarely colocalized with SOX2 and Ki-67 .
The neonatal mice received stereotactic injection of 50 nL recombinant adeno-associated virus 9 (rAAV9). (A, B) The eGFP-positive cells were detected from P1 to P5. Scale bar = 50 μm. The number of positive cells was counted and data are presented as the mean ± standard deviation of three independent experiments (n = 3). ***$P < 0.001$ versus the P1 group. Following intracerebral injections of AAV9 in neonatal mice, the numbers of SOX2 (C), Ki-67 (E), and DCX (G) positive cells were assessed through immunostaining at 5 days post-injection. The magnified view of the boxed region is shown on the right. Scale bar = 50 μm. (D, F, and H) Data are presented as the mean ± standard deviation of three independent experiments (n = 3). P1, postnatal at day 1; P2, postnatal at day 2; P3, postnatal at day 3; P4, postnatal at day 4; P5, postnatal at day 5.

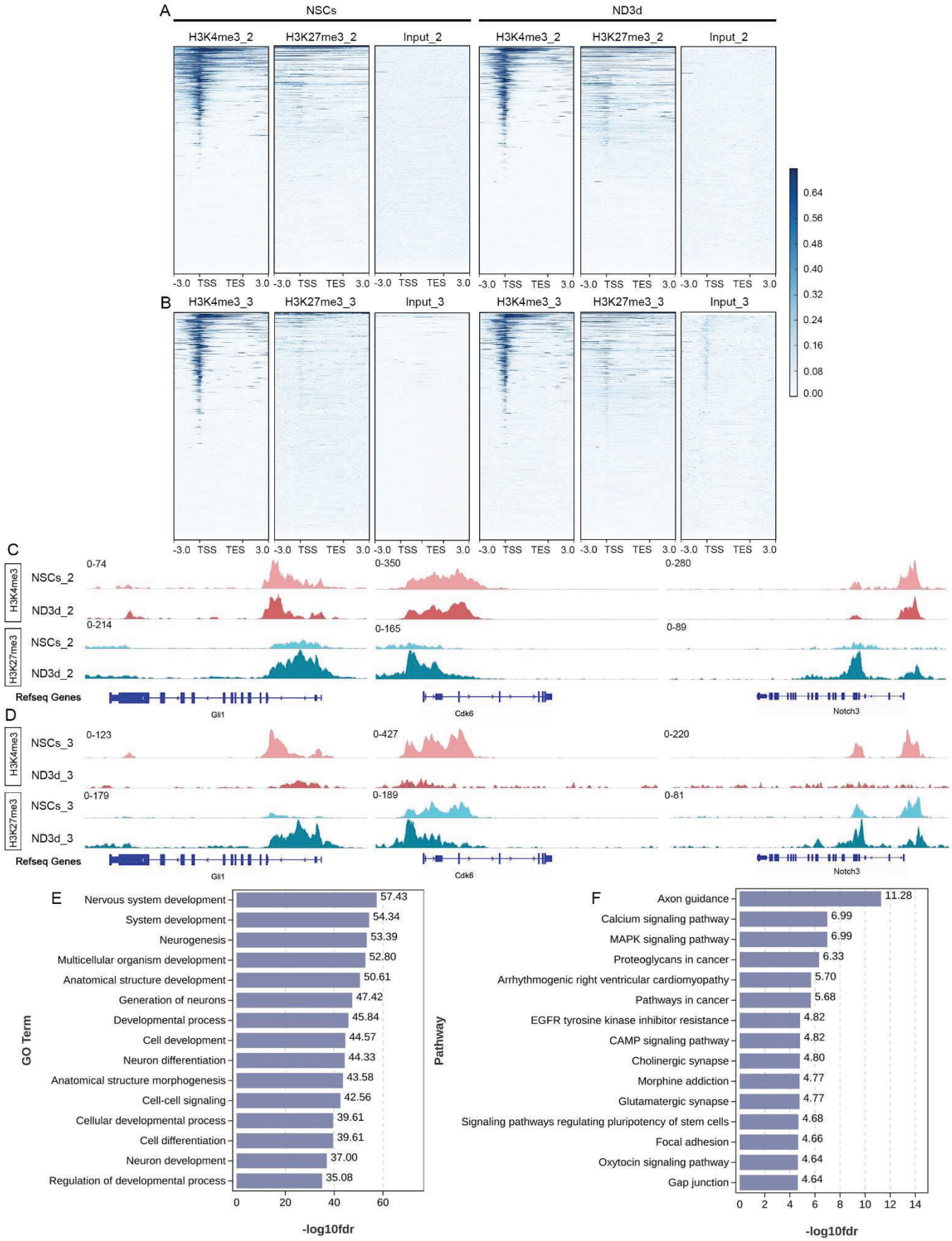


Supplement Figure 7. The effect of H3K4me3 and H3K27me3 on NSCs proliferation and neuronal differentiation during dental gyrus development

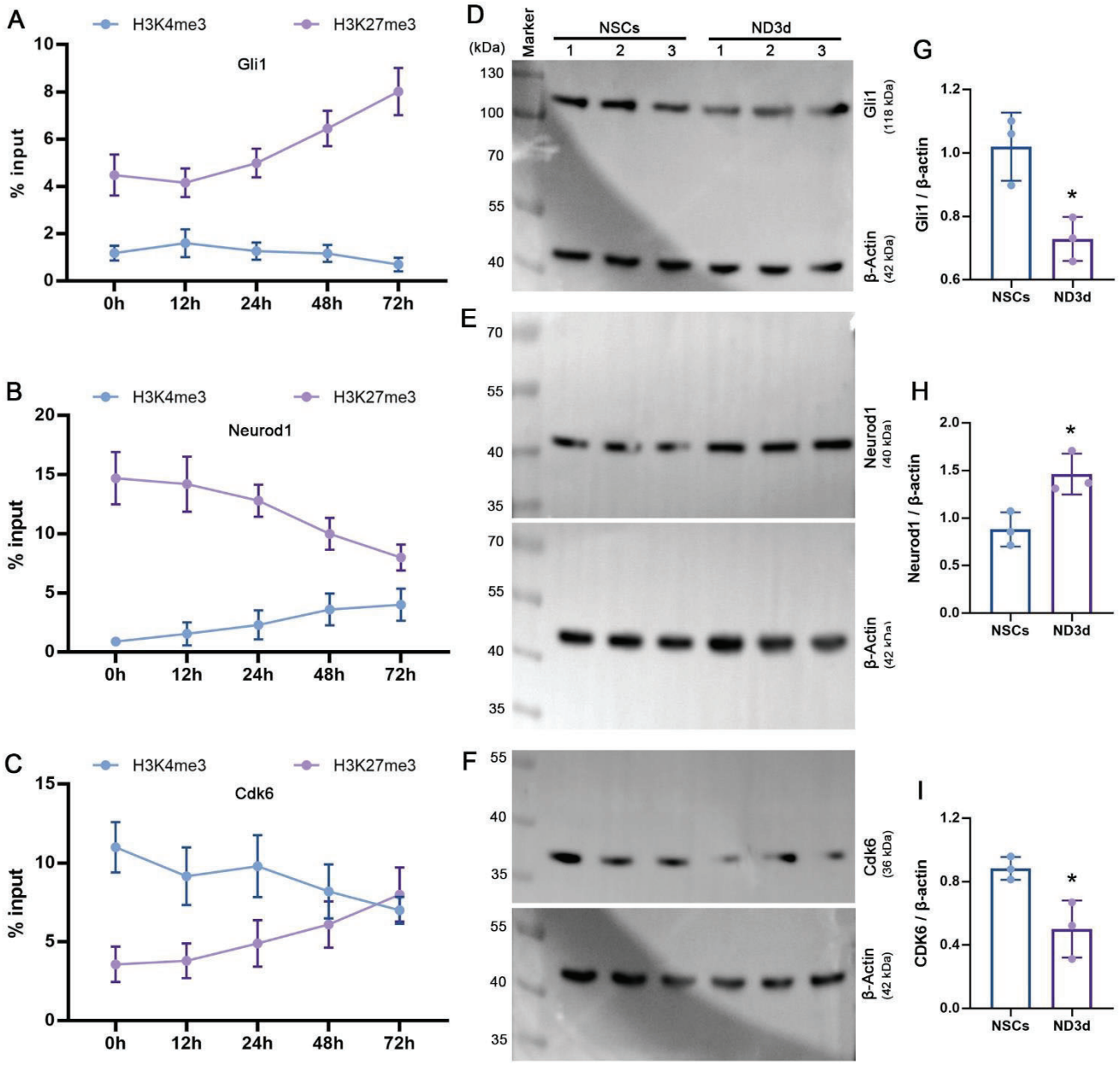
Following intracerebral injections of 50 nL rAAV9 into neonatal mice, the counts of Ki-67 (A), SOX2 (C) at P10, DCX (E) at P30, and NeuN (G), CaMKII (I) at P60 were determined. The magnified view of the boxed region is shown on the right. Scale bar = 50 μm. (B, D, F, H and J) Data are presented as the mean ± standard deviation of three independent experiments (n = 3). P10, postnatal at day 10; P30, postnatal at day 30; P60, postnatal at day 60.



Supplement Figure 8. Alterations of H3K4me3 and H3K27me3 levels do not induce apoptosis during dental gyrus development
Neonatal mice were administered a stereotactic injection of 50 nL rAAV9. Apoptotic cells were identified using the TUNEL assay at P10 (A), P30 (C), and P60 (E). Scale bar = 50 μm. (B, D and F) Data are presented as the mean ± standard deviation of three independent experiments (n = 3). P10, postnatal at day 10; P30, postnatal at day 30; P60, postnatal at day 60.



Supplement Figure 9. H3K4me3 and H3K27me3 participate in the regulation of neural development by forming bivalent domains. Neonatal mouse hippocampal NSCs were cultured in the neuronal differentiation medium for 3 days (ND3d) and ChIP-seq was preformed to profile of H3K4me3 and H3K27me3 in both NSCs and ND3d cells (immature neurons). (A, B) Heatmap of H3K4me3 and H3K27me3 ChIP-seq signals across ±3 kb from transcription start site (TSS) and transcription end site (TES). (C, D) The IGV visualization displays the ChIP-seq tracks for H3K4me3 and H3K27me3 at the *Gli1, Cdk6* and *Notch3* gene loci. ChIP-seq and RNA-seq were performed in NSCs and ND3d cells. Gene Ontology (GO) enrichment analysis (E) and KEGG pathway analysis (F) were subsequently carried out on the co-regulated genes identified in both ChIP-seq and RNA-seq datasets.



Supplement Figure 10. Changes in the ratio of H3K4me3 to H3K27me3 affect the expression of genes related to neural development
Neonatal mouse hippocampal NSCs were cultured in the neuronal differentiation medium for 3 days (ND3d). (A-C) ChIP-qPCR was used to measure the H3K4me3 and H3K27me3 enrichment in *Gli1, Neurod1,* and *Cdk6* gene at different time points during neuronal differentiation. (D-I) Western blot showing changes in Gli1, Neurod1, and Cdk6 expression. β-actin was the reference protein and the presented data were from three independent experiments (n = 3) and were expressed. **P* < 0.05 versus the NSCs group.